1: J Exp Med 1996 Mar 1;183(3):867-78 Related Articles, Books, LinkOut

# Signaling through the lymphotoxin beta receptor induces the death of some adenocarcinoma tumor lines.

**Browning JL, Miatkowski K, Sizing I, Griffiths D, Zafari M, Benjamin CD, Meier W, Mackay F.**

Department of Immunology and Inflammation, Biogen, Cambridge, Massachusetts 02142, USA.

Surface lymphotoxin (LT) is a heteromeric complex of LT-alpha and LT-beta chains that binds to the LT-beta receptor (LT-beta-R), a member of the tumor necrosis factor (TNF) family of receptors. The biological function of this receptor-ligand system is poorly characterized. Since signaling through other members of this receptor family can induce cell death, e.g., the TNF and Fas receptors, it is important to determine if similar signaling events can be communicated via the LT-beta-R. A soluble form of the surface complex was produced by coexpression of LT-alpha and a converted form of LT-beta wherein the normally type II LT-beta membrane protein was changed to a type I secreted form. Recombinant LT-alpha 1/beta 2 was cytotoxic to the human adenocarcinoma cell lines HT-29, WiDr, MDA-MB-468, and HT-3 when added with the synergizing agent interferon (IFN) gamma. When immobilized on a plastic surface, anti-LT-beta-R monoclonal antibodies (mAbs) induced the death of these cells, demonstrating direct signaling via the LT-beta-R. Anti-LT-beta-R mAbs were also identified that inhibited ligand-induced cell death, whereas others were found to potentiate the activity of the ligand when added in solution. The human WiDr adenocarcinoma line forms solid tumors in immunocompromised mice, and treatment with an anti-LT-beta-R antibody combined with human IFN-gamma arrested tumor growth. The delineation of a biological signaling event mediated by the LT-beta-R opens a window for further studies on its immunological role, and furthermore, activation of the LT-beta-R may have an application in tumor therapy.

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1: J Inflamm 1995-96;46(4):220-34 

# Proinflammatory responses are efficiently induced by homotrimeric but not heterotrimeric lymphotoxin ligands.

**Hochman PS, Majeau GR, Mackay F, Browning JL.**

Biogen, Cambridge, Massachusetts 02142, USA.

The cytokine, lymphotoxin [LT, tumor necrosis factor beta (TNF beta)] is a potent mediator of proinflammatory and tumoricidal activities. Soluble lymphotoxin is a complex of three LT alpha chains. Its receptors, TNF-R55 and TNF-R75, bind in clefts formed by adjacent identical LT alpha monomers. LT also exists as membrane anchored heterotrimers comprised of LT alpha and LT beta chains. The major and minor membrane forms, LT alpha 1 beta 2 and LT alpha 2 beta 1, respectively, bind a unique receptor, LT beta-R. As LT alpha 2 beta 1 expresses an LT alpha-alpha cleft, it also binds TNF-R. In this report we have compared the effects of ligand engagement of TNF-R and LT beta-R by evaluating the ability of soluble LT alpha beta complexes to initiate activities of human umbilical vein endothelial cells which are characteristically signalled by TNF. We recently reported that soluble LT alpha 1 beta 2 signals via LT beta-R to mediate cytotoxicity of a subset of gamma interferon (IFN-gamma) treated carcinomas. We now show that human LT alpha beta heterotrimers do not efficiently activate LT beta-R+, TNF-R+ human endothelial cells in vitro and only inefficiently mediates lethal toxicity in mice. We also show that neither LT alpha beta heterotrimer signals via TNF-R; in fact LT alpha 2 beta 1 trimers fail to activate NF-kappa B and rather inhibit ligand-induced TNF-R signalling supporting the role for aggregation in TNF-R signalling. Thus, the ability of LT alpha beta complexes to efficiently initiate tumoricidal but not inflammatory activities distinguishes the LT/LT beta-R from the LT/TNF-R pathways and suggest novel strategies for exploiting the LT ligands in tumor therapy and for inhibiting TNF-R-mediated inflammatory sequellae.

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1: J Immunol 1995 Jan 1;154(1):33-46 Related Articles, Books, LinkOut

# Characterization of surface lymphotoxin forms. Use of specific monoclonal antibodies and soluble receptors.

**Browning JL, Dougas I, Ngam-ek A, Bourdon PR, Ehrenfels BN, Miatkowski K, Zafari M, Yampaglia AM, Lawton P, Meier W, et al.**

Department of Immunology and Inflammation, Biogen, Cambridge, MA 02142.

Lymphotoxin (LT) is a cytokine related to TNF, found in human systems in both secreted and membrane bound forms. The well characterized secreted form is a trimer of a single protein, LT-alpha, whereas the surface form is composed of a complex between two related molecules, LT-alpha and LT-beta. Because there is a distinct receptor for the complex, the membrane form is believed to signal via events different from those elicited by TNF and secreted LT-alpha. By using a battery of anti-LT-alpha and LT-beta mAbs, it is clear that two LT surface forms exist on the surface of PMA-activated II-23 cells, a human T cell hybridoma. Assuming that these surface forms are trimers, a minor form appears early after induction having an apparent stoichiometry of LT-alpha 2/beta 1 and is recognized by one group of anti-LT-alpha mAbs and the p55-TNF receptor. The second and predominant form has an apparent LT-alpha 1/beta 2 composition and is recognized by a second group of pantrophic anti-LT-alpha mAbs and the LT-beta receptor. Neither of the heteromeric forms nor a putative LT-beta homotrimeric form were found to be secreted. The properties of surface LT on the II-23 cell system were similar to those of the surface LT forms on Chinese hamster ovary cells transfected with both LT-alpha and LT-beta genes and a number of lymphoid tumor lines. These experiments point toward the LT-alpha 1/beta 2 complex as the predominant membrane form of LT on the lymphocyte surface, and this complex is the primary ligand for the LT-beta receptor.

PMID: 7995952 [PubMed - indexed for MEDLINE]

1: J Immunol 1997 Oct 1;159(7):3299-310 

# Cytotoxic activities of recombinant soluble murine lymphotoxin-alpha and lymphotoxin-alpha beta complexes.

**Mackay F, Bourdon PR, Griffiths DA, Lawton P, Zafari M, Sizing ID, Miatkowski K, Ngam-ek A, Benjamin CD, Hession C, Ambrose CM, Meier W, Browning JL.**

Department of Immunology, Biogen, Cambridge, MA 02142, USA.

Human lymphotoxin-alpha (LT alpha) is found in a secreted form and on the surface of lymphocytes as a complex with a second related protein called lymphotoxin-beta (LT beta). Both secreted human LT alpha and TNF have similar biological activities mediated via the TNF receptors, whereas the cell surface LT alpha beta complex binds to a separate receptor called the LT beta receptor (LT beta R). The murine LT alpha and LT beta (mLT alpha and mLT beta) proteins have never been characterized. When recombinant mLT alpha was produced by either of several methods, the protein had a very low specific activity relative to that of human LT alpha in the conventional WEHI 164 cytotoxicity bioassay. The weak activity observed was inhibited by a soluble murine TNF-R55 Ig fusion protein (mTNF-R55-Ig), but not by mLT beta R-Ig. Coexpression of both mLT alpha and a soluble version of mLT beta in insect cells led to an LT alpha beta form that was cytotoxic in the WEHI 164 assay via the LT beta R. To determine whether natural mLT alpha-like forms with cytotoxic activity comparable to that of secreted human LT alpha were secreted from primary spleen cells, splenic lymphocytes were activated in various ways, and their supernatants were analyzed for cytotoxic activity. Using specific Abs to distinguish between mTNF and mLT, a TNF component was readily detected; however, there was no evidence for a secreted mLT alpha cytotoxic activity using this assay. Combined, these observations suggest that secreted mLT alpha may not play a role in the mouse via interactions with TNF-R55, and the ramifications of this hypothesis are discussed.

PMID: 9317128 [PubMed - indexed for MEDLINE]

1: Immunity 1997 Apr;6(4):491-500 Related Articles, Books, LinkOut



# Distinct roles in lymphoid organogenesis for lymphotoxins alpha and beta revealed in lymphotoxin beta-deficient mice.

**Koni PA, Sacca R, Lawton P, Browning JL, Ruddle NH, Flavell RA.**

Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06520, USA.

Lymphotoxin alpha (LT alpha)-deficient mice revealed critical roles for LT alpha in lymphoid organogenesis, but it is not clear whether LT alpha functions through an LT alpha homotrimer (LT alpha3) or LT alpha/beta heterotrimers. We generated LTbeta-deficient mice and found them to lack Peyer's patches, peripheral lymph nodes, splenic germinal centers, and follicular dendritic cells. Unlike LT alpha-deficient mice, LT beta-deficient mice had cervical and mesenteric lymph nodes. Furthermore, the mesenteric lymph nodes had germinal center-like regions, although these structures appeared to lack follicular dendritic cells. The absence of cervical and mesenteric lymph nodes in LT alpha-deficient mice, and yet their presence in LT beta-deficient mice and in mice deficient in tumor necrosis factor receptor types I and II, suggest that LT alpha3 may signal via an as yet unidentified receptor.

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